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PRINCIPAL INVESTIGATOR: Ann E. Allworth, Ph.D.

CONTRACTING ORGANIZATION: Howard University
Washington, DC 20059

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6. AUTHOR(S):
Ann E. Allworth, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Howard University
Washington, DC 20059

E-Mail: aallworth@howard.edu

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13. ABSTRACT ***(Maximum 200 Words)***

An unresolved problem in the treatment of breast cancer is the development of chemoresistance in patients who have been treated with chemotherapeutic agents. One of the most efficacious drugs in the treatment of metastatic breast cancer is cisplatin. A major goal of this project is to identify the cellular components involved in reversal of resistance induced when lanthanide metal ions are added to cisplatin treatment of resistant cultured breast cancer cells. Fluorescence microscopy was employed to visualize microtubules, centrosomes, p53 and chromatin in cells treated with lanthanide metals alone or in combination with cisplatin. The results indicate that terbium and gadolinium ions shift tubulin equilibrium toward microtubule polymerization, an effect seen in cells treated with taxol. In resistant cells allowed to recover, addition of either ion disrupted the structural integrity of the centrosome, a phenotype seen in sensitive cells that had been treated with cisplatin. The tumor suppressor protein p53 was localized in the nucleus of all treatment groups. Importantly, when either ion was added to cisplatin treatment of resistant cells and these cells were allowed to recover for 48 hours a higher concentration of p53 was observed, suggesting p53 involvement in resistance reversal. Finally, nuclei of cells responding to cisplatin treatment displayed morphology characteristic of apoptosis. Collectively these results support the hypothesis that either terbium or gadolinium ions, when added to cisplatin treatment of resistant cells, activate a yet to be identified kinase that phosphorylates bcl-2 directing cells to the apoptotic pathway and effectively reversing resistance to cisplatin in these cells.

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INTRODUCTION

This propsal was an innovative endeavor to unravel the intracellular signalling events that occur when terbium ions (Tb^{3+}) or gadolinium ions (Gd^{3+}) are added to cisplatin treatment of cisplatin resistant MDA-MB231 human breast cancer cells. Preliminary data demonstrated that either trivalent lanthanide ion reverses the cellular phenotype of microtubules in resistant cells treated with cisplatin alone and mimics the phenotype seen when cisplatin sensitive MDA-MB231 cells are treated with cisplatin. These cells (which were treated for 1 hour, then allowed to recover for 72 hours) display atypical microtubule organization and do not appear to contain a centrosome.

Importantly, the acute effect of these ions on both cell types is a dramatic increase in microtubule polymerization with enhanced activity at the centrosome. This evidence overwhelmingly points to the centrosome as the intracellular site that is affected. Therefore, the proposed study included a survey of the disposition of relevant antigens to centrosomal proteins before and after exposure to treatment with either vehicle buffer alone, Tb^{3+} in buffer, Gd^{3+} in buffer, cisplatin in buffer, Tb^{3+}/cisplatin in buffer, or Gd^{3+}/cisplatin in buffer.

Additionally, because both Tb^{3+} and Gd^{3+} can substitute at cellular Ca^{2+} binding sites and alterations in intracellular Ca^{2+} levels regulate cell cycle progression, a study of intracellular changes in live cells was to be carried out using photolabile caged Ca^{2+} chelators-AM. Synchronized populations of sensitive and resistant cells were to be monitored at key cell cycle checkpoints to determine if differences in intracellular Ca fluctuations exist between control cells and those exposed to any of the treatment groups. Lastly, polyacrylimide gel electrophoresis/Western blotting was to be used to ascertain basal levels of P glycoprotein and glutathione S transferase p, enzymes frequently overexpressed in resistant cancer cells. In addition, levels of alpha, beta, and gamma tubulin expression were to be assessed before and after exposure to the treatment groups to determine whether treatments alter tubulin's basal expression in these cells. The hypothesis of this proposed study, that the centrosome/spindle pole represents an intracellular site of drug resistance, is unique and challenges existing paradigms of cellular resistance mechanisms.

BODY

As was related in the previous 2 reports, this proposal seemed to be fraught with problems outside of the control of the PI. Collectively, these included during the first year, the loss of the laboratory refrigerator with the resultant loss of supplies and downtime, as well as the inability to maintain lives cell experiments on the microscope stage with any degree of consistency. Task 1 of the proposal called for live cell experiments to monitor intracellular calcium changes, however cells could not be maintained on the microscope stage and a great deal of laboratory time was lost trying to construct makeshift chambers to do the experiments.

In year 2, progress was hampered by first a computer failure which began as minor in February of 2000, but continued until the computer failed completely in May of 2000. In addition, there were issues with determining optimal fixation methods for staining the centrosomal components. The final and most deleterious issue was that of contamination. While this problem began as sporadic, it continued and worsened.

Progress on this proposal was effectively stopped by virtue of the fact that cultures and/or experiments invariably were contaminated. This continued despite repeated thorough cleaning of all apparati associated with culturing the cells. Even when cells were not overtly contaminated, i.e. visible floating muck, after fixation and staining it was clear via the Hoechst

staining that microorganisms were present in the cultures. Not only did this make it difficult to visualize microscopic changes, it also invalidated any results that may have been observed.

Delays in this report were in the hope that success would be achieved in that I had requested from sources here that an incubator and hood be provided me outside of the common contaminated culture room, so that I could try to carry out reviewer recommended experiments needed to get the 2 papers I had submitted, published. Unfortunately the equipment came too late to accomplish this, as the grant ran out before the equipment was available. Recently, I have managed to get a small amount of monies from my department chair and hope to finish this work so that these two important papers can be published and the work that I was able to do with this DOD grant will not be lost.

KEY RESEARCH ACCOMPLISHMENTS

- Immunofluorescent staining protocol for $p34^{cdc2}$ successfully developed and implemented
- Immunofluorescent staining protocol for bcl-2 successfully developed and implemented
- Immunofluorescent staining protocol for p53 successfully developed and implemented
- Immunofluorescent staining protocol for gamma tubulin successfully developed and implemented
- Observations of changes in the staining patterns of the aforementioned antigen probes without treatment (controls), after treatment with terbium alone, gadolinium alone, cisplatin alone, a combination of terbium and cisplatin, or a combination of gadolinium and cisplatin have been made
- Observations of changes in the staining patterns of p53 after treatment with taxol alone or taxol in combination with cisplatin.

REPORTABLE OUTCOMES

- An abstract was submitted and presented at the 2000 DOD Breast Cancer meeting in Atlanta.
- Peer reviewed paper submitted and not accepted awaiting suggested experiments. Allworth, A.E. and R. Canada. Lanthanide metal ions alter the microtubule network in cultured human breast and ovarian cancer cells.
- Peer reviewed paper submitted and not accepted awaiting suggested experiments. Allworth, A.E. and R. Canada. Is the centrosome involved in the reversal of cisplatin resistance in cultured human breast cancer cells?

CONCLUSIONS

In fixed cells, it has been shown that $p34^{cdc2}$ accumulates in a cell cycle dependent manner at the spindle poles of MDA-MB 231 human breast cancer cells, which is consistent with previously published data on other cell types . Additionally, these experiments demonstrated that bcl-2 has an enhanced concentration at the centrosome and may colocalize along microtubules. This piece of data may be particularly relevant to bcl-2's role in apoptosis as well as being important to understanding how microtubule integrity or lack thereof is interfaced with the cellular decision to enter the apoptotic pathway. An interesting aspect to this result is the implications it may have for understanding the resistance mechanism that is operational in MDA-MB 231 cells.

Another important conclusion was the demonstration of increased accumulation of p53 in cisplatin treated cells which may provide a better understanding of resistance mechanisms in MDA-MB 231 cells.